ECOLOGY, KARYOLOGY, AND ANATOMY OF THE PLANARIAN *PENTACOELUM HISPANIENSE* SLUYS, 1989 (PLATYHELMINTHES, TRICLADIDA)

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ABSTRACT

On the basis of recently discovered new populations of the triclad flatworm *Pentacoelum hispaniense* new data are detailed on the ecology, reproduction, karyology, and anatomy of the species, including the first description of the external appearance of living specimens. Living animals show three refringent patches on the dorsal surface of which the nature and function remain unknown. The diploid chromosome complement consists of 14 chromosomes. The species appears to be restricted to relatively warm, lowland bodies of water, where it may be found in heterogeneous habitats. The species reproduces only sexually through the production of cocoons. This study presents the first record of a digenean flatworm parasitizing a marine triclad in the form of a metacercaria.

Key words: Platyhelminthes, Tricladida, *Pentacoelum hispaniense*, Spain, ecology, karyology, anatomy, parasite, Digenea, metacercaria.

RESUMEN

Ecología, cariología y anatomía de la planaria *Pentacoelum hispaniense* Sluys, 1989 (Platyhelmintes, Tricladida)

Basándonos en las nuevas poblaciones descubiertas del tríclado *Pentacoelum hispaniense* aportamos nuevos datos ecológicos, reproductivos, cariológicos y anatómicos de la especie, incluyendo la primera descripción de la anatomía externa. Estos animales presentan tres manchas dorsales refringentes de naturaleza y función desconocidas. El complemento cromosómico diploide consta de 14 cromosomas. La especie parece restringida a masas de agua relativamente cálidas de tierras bajas, donde puede ser encontrada en hábitats heterogéneos. Su reproducción es exclusivamente sexual mediante la producción de capullos. El presente estudio recoge la primera cita de un digenio parasitando un tríclado del grupo de las marícolas, en el estadio de metacercaria.

Palabras clave: Platyhelminthes, Tricladida, *Pentacoelum hispaniense*, España, ecología, cariología, anatomía, parásito, Digenea, metacercaria.

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Introduction

The planarian *Pentacoelum hispaniense* Sluys, 1989 belongs phylogenetically to the group of marine triclads or Maricola (Note: the original spelling of the specific epithet was corrected by Sluys and Kawakatsu, 1995). The most recent monograph on this suborder was published by Sluys (1989), while Sluys and Kawakatsu (2005) described several new species and provided a more recent review of the biodiversity and biogeography of the group.

Despite its phylogenetic affinities, *P. hispaniense* was first found in a freshwater habitat near Algemesí, NE Spain, located 15-20 km inland from the Mediterranean coast (Sluys, 1989; Sluys *et al.*, 1990). At the time, repeated attempts to find more animals were unsuccessful (M. Riutort, pers. comm.), thus restricting the amount of information on the biology of the animals that could be provided in the first accounts on this species. The recent finds of new populations allow us to considerably expand our knowledge on the ecology, reproduction, karyology, and anatomy of the species. Furthermore, we provide the first description of the external appearance of living specimens and present the first record of a digenean flatworm parasitizing a marine triclad.

Material and methods

The lower plains of the river Ebro (Fig. 1), as well as some tributaries and artificial irrigation channels, were sampled during repeated visits over several seasons in 2001-2004. One series of collections was made in June 2002 in the rivers Xúquer, Magre, and Acèquia Real, and another series in July 2004 in the rivers Xúquer, Magre, and Barranc dels Llops. Planarians were collected from under stones in fresh and brackish waters with the help of a fine paint brush. Conductivity was measured with the help of conductimeter with the sensor placed at 10 cm under the surface and approximately at the same distance from the bottom, never in direct contact with it, at an approximate distance of 1m from the shore (representative of the habitat of *P. hispaniense*). Animals from Barranc dels Llops were kept alive for further studies under laboratory conditions (13º, 17º, and 22ºC, thus trying to determine the best culturing conditions) in water from the original habitat and were fed with veal liver once or twice a week. Specimens stored at 17º died in a short period of time, most likely because of a fungal infection. Animals for histological studies were killed using Steinmann's fluid (cf. Sluys, 1989) and subsequently fixed in 70% ethanol. Fixed specimens were embedded in paraffin, cut at intervals of 5-7 µm, and mounted on gelatine-coated slides. After deparaffination in xylene and dehydration through an ethanol series, the sections were stained in Harris haematoxylin (cf. Humason, 1967) and mounted in DePeX. Histological preparations of voucher specimens are deposited in the Zoological Museum Amsterdam (ZMA).

For karyological analyses, minimally 8 individuals were examined from three populations: Riu Magre, Móra d'Ebre and Deltebre. The limited abundance of adults and the very small size of the chromosomes in the species (which make them very difficult to measure) prevented us obtaining karyological information from all the studied populations. The hatchlings obtained in the laboratory were not used for this purpose because they are too small and we have not been able to rear adults from them. Three metaphasic plates from 3-4 days regenerating blastemas at 17ºC were obtained for each population; regenerating animals were kept in 0,05% colchicine-water solution during six hours. Tissues were fixed in methanol-acetic acid (3:1), dissociated in 50% ice-cold acetic acid, and poured onto glass slides at 65ºC. Subsequently, the tissues were dried, stained in a Giemsa-water solution (1:20) for 10 minutes, washed with water, and mounted in DePeX. Relative length was calculated as chromosome length x 100/total length of haploid complement. Centromeric index was calculated as length of short arm x 100/total length of chromosome (Levan *et al.*, 1964).

Information on the number of hatchlings was obtained from cocoons collected in the field from Deltebre, Ullals de Baltasar, River Magre, Barranc de Llops and River Xúquer and subsequently maintained in the laboratory at 13ºC. The same information was obtained at 22ºC for the Barranc dels Llops population. Dimension of the cocoons and area of body surface of the hatchlings was obtained from cocoons collected in the field from Deltebre and Ullals de Baltasar and subsequently maintained in the laboratory at 13ºC. Cocoon diameter and body surface area of the hatchlings were determined on the basis of digital photos; the images were analyzed with the Image J program. Data on cocoon size include measurements taken on non-viable cocoons. Data on time of emergence and number of hatchlings per cocoon from the river Barranc dels Llops were obtained from cocoons laid under laboratory conditions (13 ± 1ºC and 22 ± 1ºC) after 15-20 days of acclimatization of the adults at these temperatures.

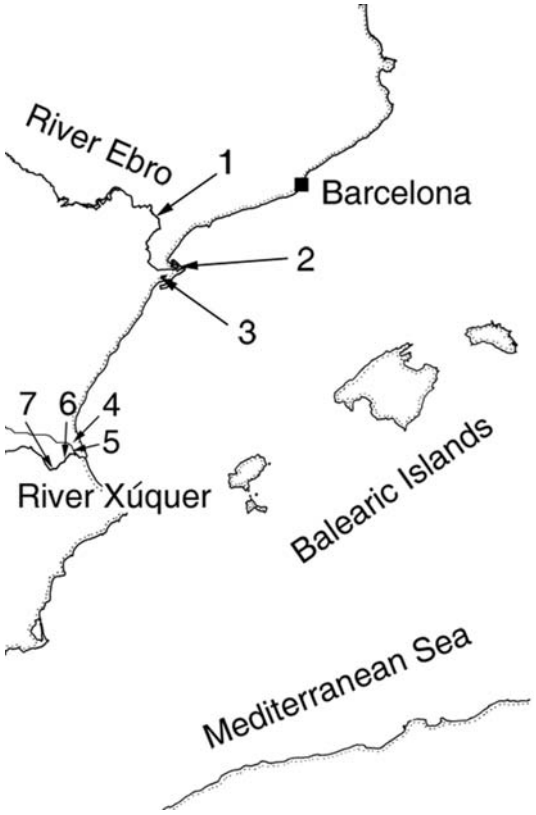

Fig. 1.— Sampling localities for *Pentacoelum hispaniense.* 1, Móra d'Ebre; 2, Deltebre; 3, Ullals de Baltasar; 4, Acèquia Real (probably in proximity of the type locality); 5, Riu Magre; 6, Antella; 7, Barranc dels Llops.

Fig. 1.— Localidades muestreadas con poblaciones de *Pentacoelum hispaniense.* 1, Móra d'Ebre; 2, Deltebre; 3, Ullals de Baltasar; 4, Acèquia Real (probablemente cerca de la localidad tipo); 5, Riu Magre; 6, Antella; 7, Barranc dels Llops.

Metacercariae living in specimens of *P. hispaniense* were obtained by disintegrating the planarians with the help of a fine brush. The metacercariae were then pipeted into an almost boiling saline solution (concentrations in g/l: NaCl, 9,88; KCl, 0,37; $CaCl_2$, 0,33; $MgCl_2$, 0,14; Na_2HPO_4, 0,17; NaH_2PO_4, 0,04; pH: 7,2) and subsequently preserved in 70% alcohol. Sporocysts from the digenean in *Melanopsis dufouri* Férrussac, 1823 were obtained from living snails by cracking the shells. Fixation of the sporocysts followed the same procedure as for the metacercariae. Salinity of the habitat at each locality was measured with a conductimeter between the last week of June and the first week of July 2002.

Fig. 2.— *Pentacoelum hispaniense*. Living animal. Scale bar: 500 μm.

Fig. 2.— *Pentacoelum hispaniense*. Ejemplar vivo. Escala: 500 μm.

Results

Systematic section

Order Tricladida Lang, 1884
Suborder Maricola Hallez, 1892
Family Bdellouridae Diesing, 1862

Pentacoelum hispaniense Sluys, 1989

Material examined. ZMA V.Pl. 6588, Ullals de Baltasar, Ebro Delta, Spain, 21 November 2002, sagittal sections on 1 slide; V.Pl. 6589, ibid., 20 November 2002, transverse sections on 3 slides; V.Pl. 6590, ibid., 26 November 2002, sagittal sections on 2 slides; V.Pl 6591, ibid., 10 December 2002, transverse sections on 2 slides.

Description. Since earlier papers on the species (Sluys, 1989; Sluys *et al.*, 1990) have adequately detailed the morphology of the copulatory apparatus and other anatomical structures, we will here only

Table 1.— Mean cocoon size in millimetres (mm) [in brackets, number of cocoons analyzed], body surface area of the hatchlings (mm^2) [in brackets, number of hatchlings analysed], number of hatchlings per cocoon (n), and total hatchling body surface per cocoon (s.c.=mm^2 x n) from two populations collected in October 2004 and maintained at 13ºC.

Tabla 1.— Tamaño medio (mm) de los capullos (entre paréntesis, número de capullos analizados), área de la superficie corporal de los neonatos (mm^2) (entre paréntesis, número de neonatos analizados), numero de neonatos por capullo (n) y superficie total de neonatos por capullo (s.c= mm^2 x n) de dos poblaciones recolectadas en octubre de 2004 y mantenidas a 13ºC.

	mm	mm^2	n	s.c.
Deltebre	0.62 (50)	0.324 (76)	4.57	1.48
Ullals de Baltasar	0.47 (42)	0.301 (39)	2.24	0.67

make note of those traits that were not recorded previously. Living animals (Fig. 2) are maximally up to 6 mm long and 1.4 mm wide but usually much smaller (3-4 mm). The anterior end is broadly rounded and the posterior end is slightly forked, albeit that this forked tail is only clearly visible under observation through a hand lens or dissecting microscope. The dorsal body surface presents brown pigmentation but still allows one to see the intestinal branches. There are three refringent patches on the dorsal side: one anterior to the eyes, one in the middle of the body, and a third one at the tail. Occasionally other patches may occur. These patches are brown in transmitted light, but become refringent in reflected light. This effect is observable under laboratory conditions but is clear also in the field, with only the help of sunlight. There is also a distinctly pale dorsal line that is not refringent. Eyes are set in pigment free patches. The eye shows a thick corneal membrane (Fig. 4), with a thickness of about 4 µm. The ventral surface is not pigmented, but it is possible to observe two white lateral spots at the level of the copulatory apparatus, probably corresponding to the lateral bursae. In some individuals lateral bursae may be absent or be reduced to a simple channel running from the ventral body surface to the oviduct. In the Ullals' population the last-mentioned condition seems to be the maximum developmental stage since no animals with complete lateral bursae were found. The posterior gut trunks meet in the posterior end of the body, as is the case also in the congeners *P. fucoideum* Westblad, 1935 and *P. punctatum* (Brandtner, 1935).

Table 2.— Mean and standard deviations of the number of hatchlings per cocoon (range in brackets), and the number of cocoons analyzed (n) from five populations, including data on cocoons deposited and developed completely under laboratory conditions at two different temperatures.

Table 2.— Media y desviación estándar del número de neonatos por capullo (rango entre paréntesis), y número de capullos analizados (n) de cinco poblaciones, se incluyen datos de capullos depositados y desarrollados completamente en condiciones de laboratorio a dos temperaturas diferentes.

Locality	Date	x ± s.d.	n
Deltebre	November 2003	4.33 (2-6) ± 1,15	12
	July 2004	3.75 (1-7) ± 1,66	12
	October 2004	4.57 (1-7) ± 1,67	42
Ullals de Baltasar	November 2002	2.78 (1-5) ± 1,09	9
	November 2003	2.88 (1-6) ± 1,19	24
	October 2004	2.24 (1-6) ± 1,16	25
River Magre	June 2001	3.73 (1-7) ± 1,62	11
Barranc dels Llops	July 2004	3.98 (1-7) ± 1,75	81
	lab. 13ºC	2.44 (1-5) ± 1,00	45
	lab. 22ºC	1.93 (1-4) ± 0,70	55
Xúquer	July 2004	3.75 ± 2.5	4

The oval shaped cocoons are laid under stones. Their size varies between 0.4 and 0.7 mm. In contrast to most other species of marine triclad, old cocoons do not attain a very dark brown color but remain orange, thus allowing the observation of their contents. The maximum diameter of the cocoon can get up to 0.7 mm and varies between 0.5-0.69 mm in Deltebre and 0.38-0.57 mm in the Ullals de Baltasar (Table 1). Hatchlings emerging from these cocoons have approximately the same body surface area in both localities, viz. about 0.300 mm^2 (Table 1). For the various populations examined we determined that the average number of young hatching from a single cocoon varied between 2.78 and 4.33 under field conditions (Table 2). This number dropped to 2.44 and 1.93 in cocoons from the Barranc dels Llops population that developed completely under laboratory conditions at 13ºC and 22ºC, respectively. The mean number of days between deposition and eclosion is 40 ± 11.7 days (47 cocoons analyzed) at 13ºc and 14.1 ± 2.4 days (57 cocoons analyzed) at 22ºC (Fig. 3). A polygonal rupture allows the young planarians to hatch from their cocoons. Hatchlings are unpig-

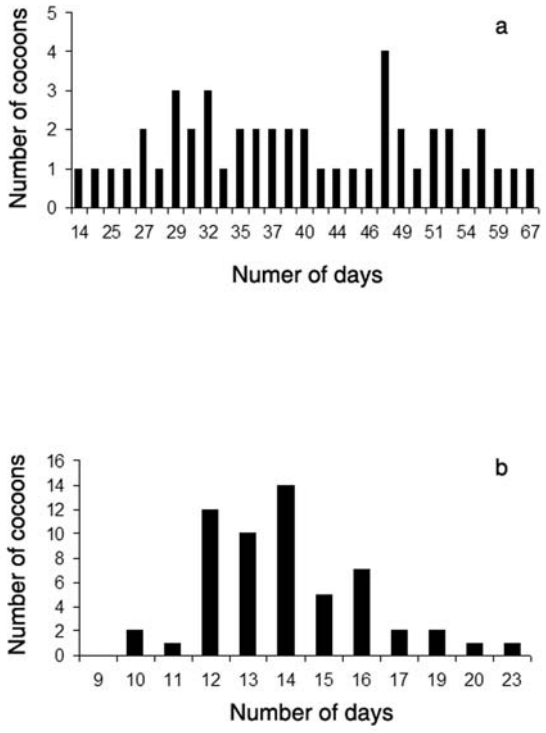

Fig. 3.— Frequency distribution of the number of days between deposition and eclosion at 13ºC (a) and 22ºC (b) of cocoons from animals collected in July 2004 from Barranc dels Llops.

Fig. 3.— Distribución de frecuencias del número de días entre la puesta y la eclosión a 13ºC (a) y 22ºC (b) de los capullos depositados por animales recolectados en el Barranc de Llops en Julio del 2004.

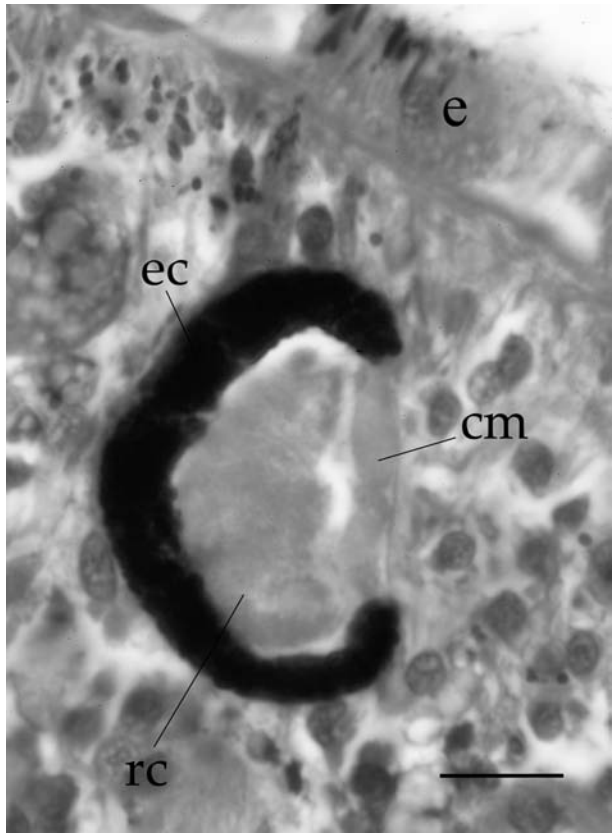

Fig. 4.— Eye structure of *P. hispaniense* (e: dorsal epidermis; ec: eye cup**;** rc: retinal cell; cm: corneal membrane). Scale bar: 10 μm.

Fig. 4.— Estructura del ojo de *P. hispaniense* (e: epidermis dorsal; ec: copa pigmentaria; rc: célula retinal; cm: membrana córnea). Escala: 10 μm.

mented, except for the refringent zones, which are visible even when they are still inside the cocoon.

KARYOLOGY. Animals from the rivers Magre, Deltebre, and Móra d'Ebre all show the same karyotype of 2n= 14, n= 7. The submetacentric chromosomes number 1 and 2 are easy to distinguish from the others by their larger size, exceeding more than two times that of the shortest chromosomes. The centromeric index of number 3 remains undetermined. In most of the mitotic plates chromosome 3 overlapped with other chromosomes, thus preventing a clear view of the position of the centromere and the calculation of its centromeric index in a significant number of plates (the few non-overlapping chromosomes were subtelocentric or telocentric). Chromosomes 4 and 5 are similar in size but easy to distinguish because number 4 is metacentric and number 5 telocentric. Chromosomes 6 and 7 are also very similar in size but number 6 is metacentric, whereas chromsome 7 is telocentric (Fig. 5; Table 3).

ECOLOGY. General information on each locality is summarized in Table 4. *P. hispaniense* was abundant in June 2000 in the river Magre, near Algemesí (15-20 km from the Mediterranean Sea), at the point where the Magre meets the river Xúquer; however, at the same locality the species was absent in July 2004. Here the species coexists with the freshwater planarian *Girardia tigrina* (Girard, 1850). *Pentacoelum hispaniense* also occurs in the Acèquia Real, an artificial irrigation channel with almost no vegetation, where it is very scarce. In the Acèquia Real *P. hispaniense* coexists with the freshwater tri-

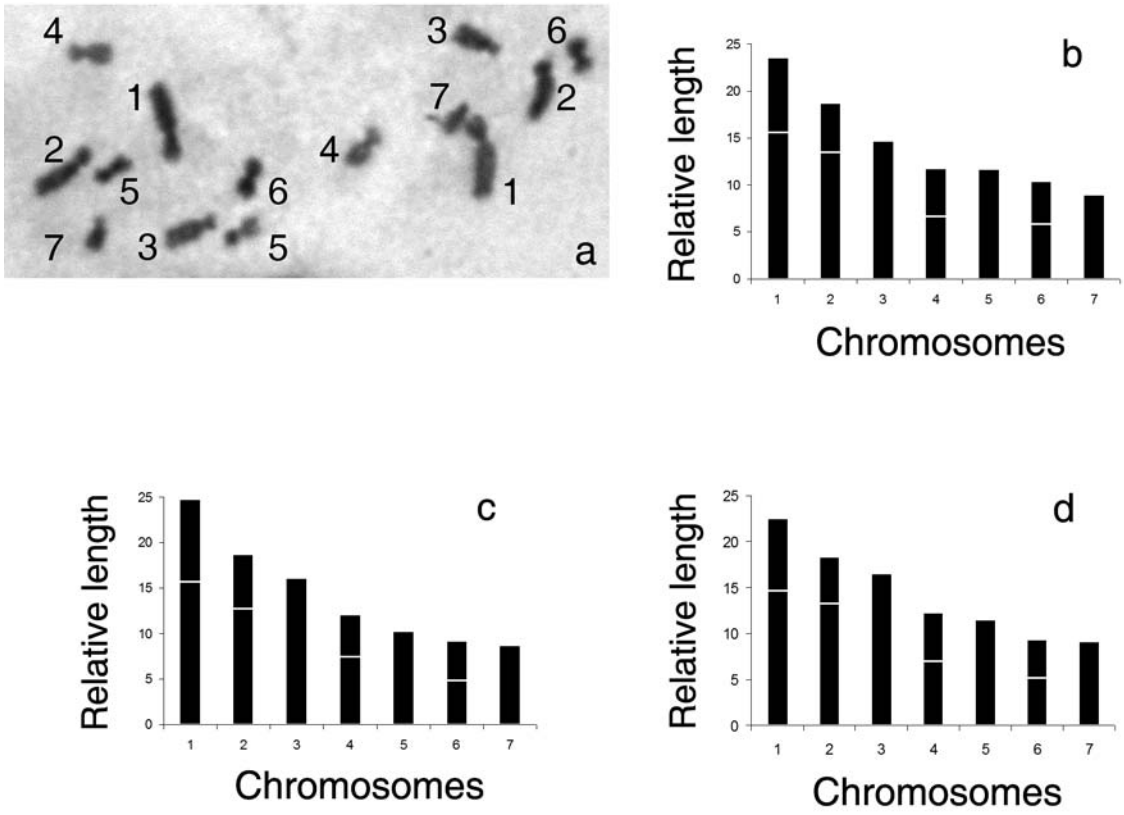

Fig. 5.— a: Mitotic metaphase plate of *Pentacoelum hispaniense* from river Magre, with 14 bivalents. Numbers indicate position of the chromosomes in the correspondening idiogram. b, c, d: Idiograms of chromosomes from three populations (b: river Magre, c: Deltebre, d: Móra d'Ebre,). For the third chromosome pair only relative length is indicated and not the centromeric index.

Fig. 5.— a: Placa metafásica de *Pentacoelum hispaniense* del río Magre, con 14 bivalentes. Los números indican la posición de los cromosomas en el idiograma correspondiente. b, c, d: Idiogramas de los cromosomas de tres poblaciones (b: río Magre, c: Deltebre, d: Móra d'Ebre,). Para el tercer par de cromosomas sólo se señala la longitud relativa y no el índice centromérico.

clads *Schmidtea polychroa* (Schmidt, 1861), *G. tigrina*, and *Dugesia* sp. The species occurs in the river Xúquer in Antella, coexisting here with *G. tigrina* and *Dugesia* sp. In the same basin, *P. hispaniense* also occurs in the river Barranc dels Llops (32 km from the Mediterranean Sea), where it is found very often together with *Dugesia* sp.

At each of the various localities in the Ebro River the situation is different. The locality Móra d'Ebre is about 22.5 km from the sea. With a width of about 50 m meters and a flow of over 8000 Hm^3/year, *P. hispaniense* is here mainly confined to shallow patches and is less abundant than other species of planarian, such as *S. polychroa*, *Dugesia sicula* Lepori, 1948, and *G. tigrina*. In point of fact, we failed to find *P. hispaniense* at this locality in 2003, while its status in 2004 is uncertain since the high water level made many sampling localities inaccessible. In 2005 the species was found again at Móra d'Ebre.

The Deltebre locality, in the delta of the Ebro river at 6 km from the sea, has the same planarian species as Móra d'Ebre, but all of them are really scarce, probably due to the brackish nature of this section of the river (conductivity 2210 μS). In this part of the river in the delta of the Ebro, there is a sea-water current along the bottom, covered by an upper layer of freshwater that during summer can be as thin as 30 cm. *P. hispaniense* lives in this upper layer of water. From December to February (when higher conductivities occur at Deltebre) cocoon production in *P. hispaniense* almost ceases; only one adult has been found during this period.

The Ullals de Baltasar locality concerns an oligotrophic limnocren complex, 6-7 km from the sea,

Table 3.— Mean and standard deviations of the relative lengths (r.l.) and centromeric indices (c.i.) of chromosomes of three populations (River Magre, Móra d'Ebre, Deltebre).

Table 3.— Media y desviación estándar del índice centromérico (c.i.) y de la longitud relativa (r.l.) de los cromosomas de tres poblaciones (río Magre, Móra d'Ebre y Deltebre).

Population	**River Magre 2n=14**		**Móra d'Ebre 2n=14**		**Deltebre 2n=14**	
Chromosome	**r.l.**	**c.i.**	**r.l.**	**c.i.**	**r.l.**	**c.i.**
1	22.57 ± 1.78	34.95 ± 1.77	24 ± 1.1	33.73 ± 2.6	24.80 ± 1.04	36.61 ± 1.26
2	18.39 ± 0.86	28.16 ± 2.16	19 ± 1.5	27.97 ± 0.9	18.75 ± 1.00	31.77 ± 3.68
3	16.53 ± 1.12	?	15 ± 1.2	?	16.10 ± 0.44	?
4	12.32 ± 1.37	43.21 ± 2.24	12 ± 0.8	43.27 ± 3.5	12.11 ± 1.22	38.58 ± 4.87
5	11.56 ± 0.97	0	12 ± 1.5	0	10.24 ± 0.13	0
6	9.41 ± 0.94	44.98 ± 5.48	10 ± 1.1	44.22 ± 4.0	9.24 ± 1.03	47.64 ± 3.34
7	9.19 ± 0.7	0	9 ± 1.1	0	8.77 ± 0.36	0

but under marine influence (España *et al*., 1992). This locality shows the richest planarian community, in a habitat characterised by a very stable temperature of 15-23ºC throughout the year. *Pentacoelum hispaniense* lives here in artificial irrigation channels arising from the limnocrens, together with *D. sicula*, *G. tigrina, Dendrocoelum lacteum* Müller, 1774 (all three being scarce), and *Phagocata ullala* Sluys, 1995, the most abundant planarian. At this locality *P. hispaniense* is always present and sexually mature throughout the year, laying cocoons without interruption, in contrast to the river populations.

PARASITE. The Ullals' population of *P. hispaniense* is parasited by a digenean flatworm (Fig. 6). The particular trematode life cycle stage present in the planarians concerns a metacercaria (R. A. Bray, pers. comm.). The planarians seem to be widely affected by this metacercaria, albeit that this is variable in time. No clearly parasitized planarians were found in March and May 2004 or in July 2005. The parasite was abundant in October 2003; during 2004-05 we found only few metacercariae in the period September-January**.** The number of parasites per adult planarian is variable and can reach at least 8 specimens per host. It is possible to identify the presence of the parasite solely with the help of a hand lens. They appear as tiny bubbles (about 150 µm in diameter) in the dorsal parenchyma and may be present from anterior to the eyes to the hind end of the body. Since it is not possible to identify a trematode on the basis of its metacercaria, we have collected snails (hosts of many known digeneans) and fishes in an attempt to find other stages in the life cycle of the parasite. In an attempt to find the adult stage, seven juveniles of *Gasterosteus gimnurus* Cuvier, 1829, ten adults of *Gambusia affinis* Baird & Girard,1853, and eleven adults of *Carassius auratus* Linnaeus, 1758 have been checked for trematode parastites, but with negative results. We selected these fishes as possible definitive hosts first because they coexist with *P. hispaniense,* and, second, because fishes are known to host adults stages of planarian infecting trematodes (Wallace, 1941; Smith, 1968). We have also examined for parasites all species of molluscs that coexist with *P. hispaniense* and thus found three different cercariae to be present in the snail *M. dufouri.*

Discussion

Pentacoelum hispaniense appears to be restricted geographically to lowland (up to 17 m above sea level) and relatively warm water bodies on the E Iberian Peninsula that are not far away from the coast (maximum of 32 km). The species can be found in various habitats (irrigation canals, natural areas; see Table 4), where it lives under stones in shallow areas. Because of the presence of a large network of agricultural irrigation channels running from the rivers Ebro and Magre, the species may be present at other locations in NE Spain. *P. hispaniense* forms part of a planarian assemblage con-

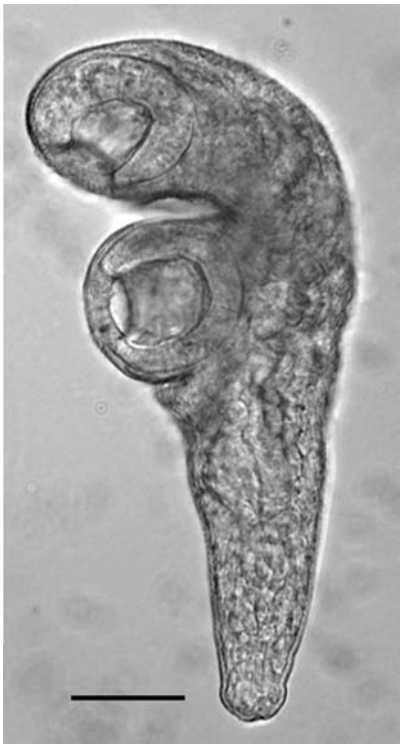

Fig. 6.— Living metacercaria. Scale bar: 10 µm.

Fig. 6.— Ejemplar vivo de la metacercaria. Escala: 10 µm.

sisting of several species, all of these being typical inhabitants of warmer waters.

In contrast to the cyclical absence of *P. hispaniense* in the river Ebro during winter, presence throughout the year of the species is observed in the Ullals de Baltasar. This difference in seasonal patterns may be attributed to differences in the water temperature during winter. At the Ullals' locality the water temperature is higher during winter, since seasonal variation in temperature, although present, is buffered as a result of the subterranean origin of the water. Temperature has been reported as an important factor in the explanation of the life history cycle and the distribution of planarians (Reynoldson *et al*., 1965; Ball & Reynoldson, 1981). We have sampled the river Ebro at upstream localities several times and have so far been unable to find *P. hispaniense*, probably because these localities have an unsuitable thermal regime, with long winters and thus prolonged periods with low water temperatures.

The Ebro delta has formed since the 14th century (Seró & Maymó, 1972), resulting from the debris carried by the river, thus forming an alluvial plain with almost no autochthonous rocky substrate. As *P. hispaniense* apparently prefers to live under stones, as is usual for many other planarians, the muddy habitat of the delta may not have provided an adequate biotope for the species. Therefore, colonization of the delta may have been facilitated by human activities creating rocky microhabitats, for example in fluvial ports. This suggests that the species is a relatively recent immigrant in the Ebro delta, using the network of irrigation channels as dispersal routes.

As is the case with other maricolans (cf. Sluys, 1989), we have not observed any sign of asexual reproduction in *P. hispaniense*, either in the field or under laboratory conditions. The species appears to reproduce only sexually, through the production of cocoons. Except for the Ullals' population, the number of hatchlings per cocoon varies between 1 and 7, with an average of about 4 hatchlings. This number of hatchlings agrees with that reported for other maricolans (cf. Sluys, 1989; Tekaya *et al*., 1999).

There was a considerable difference between the number of young hatching from cocoons in the field as compared with those kept under laboratory conditions (Table 2), with the number of hatchlings being lower in the last-mentioned case; the cause of this difference is unknown. The number of hatchlings varies from abundant (localities 2, 3, 4, and 5) to scarce (Ullals, laboratory). These sites differ in temperature regime. In the Ullals and in the laboratory the temperatures are constant or vary little, whereas at other sites the fluctuations are greater. Temperature is an important factor in the life cycle of the planarians (see below).

As found also by Reynoldson *et al*. (1965) and Tekaya *et al*. (1999), time of emergence of the hatchlings after cocoon deposition is dependent upon ambient temperature, the time period being longer at lower temperatures (Fig. 3). Although Tekaya *et al*. (1999) studied the marine planarian *Sabussowia dioica* Claparède, 1863 under somewhat different temperature conditions than we examined *P. hispaniense*,eclosion under laboratory conditions at a temperature regime of about 22ºC took place after 13 and 14 days after deposition of the cocoons by *Sabussowi*a. The *Pentacoelum* population from the Ullals' differs from the others in a considerably lower number of young hatching from a cocoon (Table 2). In comparison with animals from Deltebre the cocoon diameter of the Ullals' animals is also lower, but the size of the hatchlings, measured as body surface area, is approximately the same (Table 1). Furthermore, during October cocoon investment is lower in the Ullals' population, as compared with

Table 4.— Geographical position and some physical data of the sampling localities.

Table 4.— Localización geográfica y algunos factores físicos de las localidades de captura de los ejemplares.

Locality (meters above sea level)	UTM	Body of water	Conductivity (µS/cm²)	Salinity
Móra d'Ebre (15 m)	345 0251	wide river	1095	oligohaline
Deltebre (5)	345 0251	wide river	2210	mesohaline
Ullals de Baltasar (3)	245 9605	artificial irrigation channel	1825	mesohaline
Riu Magre (10)	743 2340	small river	1307	oligohaline
Acèquia Real (15)	-	artificial irrigation channel	-	-
Antella (40)	743 0828	wide river	-	-
Barranc dels llops (50)	743 0629	small river	-	-

the other populations. We will need much more data on cocoon production throughout the year to be able to determine precisely what could be the reason for this difference between the Ullals' animals and those from the other populations.

In the original description of *P. hispaniense* by Sluys (1989) no information was presented on the karyology and only preliminary data were provided by Sluys *et al*. (1990). The latter study identified the chromosome complement as 2n= 12. In contrast, the metaphasic plates examined in the present study all revealed a complement of 2n=14; n=7 (Table 3, Fig 5). Unfortunately we did not have animals from the original location at our disposal. However, the rivers Magre and Acèquia Real belong to the same drainage system, while Sluys *et al*. (1990) expressed that "… further studies are needed to validate the results and to determine the length and centromeric indices of the chromosomes."

Although the relative lengths of the chromosomes are not significantly different between populations, the centromeric index varies greatly, also for chromosome complements within the same population. Most likely, this is due to the difficulty in obtaining accurate measures of the short arms due to the very small size of the analyzed chromosomes. This applies particularly the chromosomes 4 and 6, which present the highest standard deviations (>4). Other studies (Charny *et al*. 2004 working with *D. sicula*) also reported large standard deviations (> 4) in the centromeric index for chromosome measurements within one population. In view of the situation that (a) high variability in the centromeric indices within one population has been observed previously in other triclad species and that (b) the analysis of the karyotype of *P. hispaniense* presents intrinsic difficulties (small size of the chromosomes and difficulty in obtaining specimens for new analysis), we conclude that the species presents very similar karyotypes within and between populations and that the observed differences are due to technical difficulties and therefore are not statistically significant or relevant.

A diploid complement of 12 chromosomes was reported for *Bdelloura candida* Girard, 1850, a species belonging to the same group as *P. hispaniense*, the family Bdellouridae, being the only other bdellourid for which karyological data are available. In general, information on chromosomes of marine triclads is very scarce; the few species that have been examined showed diploid complements of 12, 14, or 16 chromosomes (cf. Sluys, 1989).

Sluys & Bush (1988) described a comparatively thick corneal membrane or lens-like structure for *P. punctatum*. Following up on several earlier workers, Sluys (1989) observed that among the maricolans the corneal membrane of *B. candida* is developed to the greatest extent. Whether this feature of a thick corneal membrane is restricted to a particular group of bdellourids remains to be examined. But it is interesting to note that in the phylogeny of the Bdellouridae presented by Sluys (1989, fig. 303), the genus *Pentacoelum* Westblad, 1935 forms the sister group of a monophylum containing the genera *Bdelloura* Leidy, 1851 and *Syncoelidium* Wheeler, 1894.

The presence of a digenean parasiting on planarians has been recorded previously for freshwater triclads: the larval stage of the digenetic fluke *Lissorchis mutabile* (family Lissorchiidae), the adult a fluke being a parasite of fish (Wallace, 1941; Smith, 1968); five different cercariae parasit-

ing *D. lacteum*, *S. lugubris* (Schmidt, 1861), and *Polycelis nigra* (Müller, 1774) (Pike 1968); the larva of *Asymphylodora tincae* (Modeer, 1790) in *Planaria* sp. and, *Dendrocoelum* sp. (cf. Carrère, 1937 in Yamaguti, 1971). Our paper describes the first record of a digenean parasitizing a marine planarian. Since it is not yet possible to identify the metacercaria living in *P. hispaniense*, we have initiated a molecular study to compare this parasite with those present in the snail *M. dufouri*.

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